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# Augmentation of gonadotropin receptors in rat testis after hemicastration around the time of puberty

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## Abstract

Male rats were unilaterally castrated at 20, 30, or 40 days of age. Thirty days after hemicastration, the remaining testis was removed. Fresh testis weight was not significantly affected by hemicastration. LH and FSH receptors were partially purified for high-sensitivity assay. An LH receptor assay using the Leydig cell fraction showed that specific binding in rats hemicastrated at 20 and 30 days old was greater by 1.3 times than in control rats of the same age given a sham operation. There was no change in the dissociation constant of LH receptors at each age level between hemicastrated and control rats. An FSH receptor assay using the seminiferous tubule fraction showed that there was no difference in specific binding between hemicastrated and control rats at each age level. Our previous experiments suggested that the total number of Leydig cells per testis is not significantly affected by age or hemicastration and that Leydig cell size is not affected by either factor in 20- and 30-day-old rats. These results suggest that a compensatory increase in serum FSH concentration after hemiorchidectomy acts synergistically on LH-mediated testosterone synthesis in the testis and increases the number of LH receptors per Leydig cell.

## Introduction

Compensatory hypertrophy of the remaining testis occurs after hemicastration in rats[1)2)]. The magnitude of this response depends on the age at hemicastration. No increase in the weight of the remaining testis is evident in rats hemicastrated at 20 days old or later[3)4)]. Puberty in male rats starts at around 30 days old[5)]. Although there is no corresponding increase in testis weight, hemicastration of adult rats increases the Leydig cell volume in the remaining testis[6)7)]. Leydig cell numbers do not change in either prepubertal or adult rat testes following hemicastration[6)7)]. An elevation in serum follicle-stimulating hormone (FSH) concentrations occurs in hemicastrated rats[2)3)7-10)], and the levels of serum testosterone and dihydrotestosterone show age-related differences in rats hemicastrated around the time of puberty[7)]. However, serum luteinizing hormone (LH) concentrations do not change after hemicastration[7)]. Furthermore, luteinizing hormone receptors do not increase in number in adult rat after hemicastration[11)12)]. The numbers of LH and FSH receptors and testis weight are increased in prepubertal hemicastrated rats[12)], but little is known about gonadotropin receptors in the remaining testis of pubertal rats. On the basis of these observations, gonadotropin receptors in the remaining testis may change their number or affinity after hemicastration in pubertal rats. In the present study, we analyzed the changes in testicular gonadotropin receptors in rats after hemicastration around the time of puberty.

## Materials and Methods

### Animals

We used male Wistar rats from an isolated colony

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maintained at this laboratory. The rats were given rat chow and water ad libitum. They were raised under a controlled photoperiod (12L : 12D ; light on at 0600), with the temperature maintained at 23 ± 2°C and the humidity at 60 ± 5%. This project was approved and followed the guidelines determined by the Institutional Animal Care and Use Committee (IACUC) of Tokyo Medical University.

**Treatment of Animals**

The rats were unilaterally castrated at 20, 30, or 40 days of age under ether anesthesia. The left testis was externalized by an abdominal incision, and the blood vessels and vas deferens were ligated with silk thread. The testis was removed, the epididymis with its vessels and the vas deferens were returned to the abdomen, and the incision was sutured. Control rats were given a sham operation; left testis and epididymis were externalized by an abdominal incision, then all of them were returned to the abdomen, and the incision was sutured. Thirty days after hemicastration, the rats were decapitated and their remaining testes were taken out and weighed. The receptors were partially purified for high-sensitivity analysis. The tunica albuginea was cut open and turned inside out. The testis was held by a pair of tweezers and shaken gently several times in 40 mM Tris · HCl buffer containing 5 mM $MgSO_4$ and 0.1% bovine serum albumin (BSA, pH 7.4). The buffer was then centrifuged (10,000×g, 10 min), and the precipitate (Leydig cells) was used for the LH receptor assay. The remaining tissue (seminiferous-tubule-rich fraction) was removed with tweezers and used for the FSH receptor assay. This fraction was minced. Each fraction was homogenized with the buffer in a glass homogenizer with a loosely fitted Teflon pestle. The homogenates were filtered through a nylon mesh with a pore size of 250 μm. The concentration of the homogenate was adjusted to contain the extract of 200 g of fresh testes per ml in each age group. The filtrates were centrifuged for 10 min at 10,000×g. The resulting pellets were washed twice and suspended in buffer. During all procedures, the preparations were kept at 4°C. The suspensions were stored at −80°C until use. Just before use, the frozen suspensions were thawed, diluted with buffer, homogenized with the Teflon pestle homogenizer, and filtered through the nylon mesh. The protein concentrations of the pellets were determined by the method of Lowry et al.[13], employing BSA as standard.

**Iodination**

NIADDK-rat LH(rLH)-I-8 and NIDDK-rat FSH(rFSH)-I-8 were radioiodinated with $^{125}I$ ($Na^{125}I$, Radiochemical Centre, Amersham, UK) by the lactoperoxidase method at 4°C for 8 s. Then 100 μl of 8% sucrose and 0.6% sodium azide in 0.05 M phosphate buffer was added to stop the reaction. Labeled hormone was partially purified on Sephadex G-75 columns. The specific activities of [$^{125}I$]iodo-FSH and [$^{125}I$]iodo-LH were 70,000 cpm/ng and 13,000 cpm/ng respectively. The iodinated hormones were stored at −20°C until use.

**Hormone binding experiments**

All reaction tubes were coated with BSA. For the LH-binding assay, receptor preparation (100 μl ; 37.5 μg protein) and [$^{125}I$]iodo-LH (50 μl ; 0.79 ng) were incubated while shaking at 37°C for 2 h, with or without unlabeled LH (50 μl ; 5 μg NIH-LH-S19). For the FSH-binding assay, receptor preparation (100 μl ; 37.5 μg protein) and [$^{125}I$]iodo-FSH (50 μl ; 0.50 ng) were incubated while shaking at 37°C for 2 h, with or without unlabeled FSH (50 μl ; 0.5 μg NIH-FSH-S21). At the end of incubation, 1 ml cold Tris · HCl buffer (0.04 M ; pH 7.4) containing 5 mM $MgSO_4$ was added to each tube, and the tubes were centrifuged at 10,000 × g for 3 min at 4°C. The pellets were washed twice with cold buffer, and the radioactivity of the resultant pellets was counted in a well type gamma-counter (ARC-600, Aloka, Tokyo, Japan). Different amounts of [$^{125}I$]-rLH or [$^{125}I$]-rFSH were incubated with or without an excess of cold NIH-LH-I-8 or cold NIH-FSH-I-8 respectively. The dissociation constant (Kd) and the number of binding sites (capacity) were determined from the Scatchard plots[14]. Straight lines were fitted to the plots by the least-squares method. Statistics for linearity, precision, and 95% confidence interval were computed according to the methods of Bliss[15].

**Statistical analysis**

All values were expressed as means±SEM. Mean differences between treatment groups were determined using Duncan's new multiple range test[16]. Mean differences between each time period were determined using Student's t-test. Significant differences were determined by analysis of variance.

## Results

Testis weight increased progressively with age ($P<0.001$) but was not significantly affected by hemicastration (Table 1). The specific binding of [$^{125}I$]iodo-rLH per equivalent protein content (37.5 μg) of Leydig cell fraction was greater in rats hemicastrated at 20 and 30 days of age than in control rats of the same ages ($P<0.05$, Table 2). The specific binding of [$^{125}I$]iodo-rFSH per equivalent protein content (37.5 μg) of seminiferous tu-

**Table 1** Fresh testis weights (rats were killed 30 days after hemicastration) (g) ($n$=10)

| Age at operation | 20 days | 30 days | 40 days |
|---|---|---|---|
| Sham operation | 1.01±0.017 | 1.32±0.020 | 1.42±0.018 |
| Hemicastration | 1.04±0.017 | 1.33±0.029 | 1.41±0.028 |

*Values are means±SEM.

**Table 2** LH and FSH specific binding (binding in cpm/μg protein)

| Age at operation | 20 days | 30 days | 40 days |
|---|---|---|---|
| LH specific binding ($n$=8) | | | |
| Sham operation | 26.27±1.52 | 23.01±1.07 | 31.25±1.31 |
| Hemicastration | 32.45±1.92[a] | 32.43±2.11[a] | 27.60±2.53 |
| FSH specific binding ($n$=6) | | | |
| Sham operation | 24.91±1.08 | 27.41±0.88 | 25.96±1.66 |
| Hemicastration | 28.42±1.47 | 25.96±1.80 | 26.68±1.03 |

*Values are means±SEM.
[a]$P$<0.05 vs. respective controls by Duncan's new multiple range test.

**Table 3** Capacities of LH receptors (f mol/μg protein) ($n$=8)

| Age at operation | 20 days | 30 days | 40 days |
|---|---|---|---|
| Sham operation | 0.0220±0.002 | 0.0181±0.001 | 0.0302±0.003 |
| Hemicastration | 0.0191±0.003 | 0.0240±0.002[a] | 0.0307±0.004 |

*Values are means±SEM.
[a]$P$<0.05 vs. respective controls by Student's t-test.

**Table 4** Dissociation constant (Kd) of LH receptors (nM) ($n$=8)

| Age at operation | 20 days | 30 days | 40 days |
|---|---|---|---|
| Sham operation | 0.132±0.012 | 0.122±0.006 | 0.129±0.012 |
| Hemicastration | 0.116±0.007 | 0.120±0.007 | 0.136±0.016 |

*Values are means ± SEM.

bule fraction was not significantly different between hemicastrated and control rats of the same ages (Table 2). A significant difference was detected in specific binding of LH by Duncan's new multiple range test, but there was no significant difference in specific binding of FSH. Therefore, further investigation was done only with the LH binding experiments. To examine the number of binding sites, we used Scatchard plot analyses of the specific binding of LH. LH capacity was increased significantly by 30% in rats hemicastrated at 30 days compared with the corresponding controls ($P$<0.05, Table 3). No difference in LH capacity between 20- or 40-day hemicastrated and control rats was observed in the Leydig cell fraction. No significant differences were detected in the Kd of LH receptors between hemicastrated and control rats in any age group (Table 4).

## Discussion

Hemicastration at 30 days of age increased the capacity of LH receptors in the remaining testis 30 days after the operation. Brown and Chakraborty[12] reported an increase in LH and FSH receptor contents in prepubertal hemicastrated rats that was related to an alteration in testis size. In the present study, testis weight did not change between hemicastrated rats and control rats given a sham operation at any age, because the age at operation was around puberty. In one of our previous reports[7], in rats hemicastrated at 20 days old, the concentration of testosterone in controls exceeded that in hemicastrated rats. However, in rats hemicastrated at 40 days old, the situation was reversed. In rats hemicastrated at 30 days old, the testosterone levels in the hemicastrated and control rats were essentially the same, and the plasma testosterone concentration was greater in both groups than in the other age groups. Plasma dihydrotestosterone was significantly higher in the 30- and 40-day hemicastrated rats than in controls but was not significantly different between the 20-day hemicastrated and control rats. The volume densities of Leydig cells in 40-day hemicastrated rats were significantly greater than in controls. The total number of Leydig cells per testis was not significantly affected by age or hemicastration, and Leydig cell size was not affected by either in 20- and 30-day-old rats. Electron microscopy indicated that the organelles responsible for producing steroids in rats hemicastrated either at 40 days old[7] or as adults[17] were more active than those in controls, but there were no differences in organelle content between rats hemicastrated at 20 and 30 days old and controls. The cytoplasmic features of Leydig cells correlated with peripheral testosterone levels.

In this study, we used a crudely purified Leydig cell fraction. Although recovery of receptors was low, the receptor assay showed high sensitivity. In rats younger than 20 days, Leydig cell have two types of receptors, but by 50 days, they have only one[18]. Specific LH bindings of Leydig cells in rats hemicastrated at 20 and 30 days old were higher than those of the controls. That means that the Leydig cells in these rats had not become hypertrophic by 30 days after the operation, although the possibility was there. An increase in LH receptors per equivalent protein was observed in rats hemicastrated at 30 days old. A progressively increasing LH receptor population in the testes of maturing rats is a major factor in the processes leading to sexual maturation[19]. These results suggest that the Leydig cells of these rats had prepared to undergo hypertrophy. The serum FSH concentration increased in hemicastrated rats but LH remained unchanged[2)7)9)10]. Certain types of Leydig cells may be a target for FSH stimulation[20], or FSH may regulate testicular steroidogenesis through Sertoli cell factors[21)22]. One possible explanation is that FSH acts synergistically on LH-mediated testosterone synthesis in the testis[23] and increases the number of LH receptors per Leydig cell. In conclusion, the capacity of LH receptors per equivalent protein was increased 30 days after hemicastration at 30 days old without an increase in testis size.

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**References**

1) Hochereau-de-Reviers MT : Action de l'hemicastration sur l'evolution du testicule restant chez le rat. Bull Assoc Anat **151**: 364-371, 1971
2) Ojeda SR, Ramirez VD : Plasma level of LH and FSH in maturing rats : response to hemigonadectomy. Endocrinology **90** : 466-472, 1972
3) Cunningham GR, Tindall DJ, Huckins C, Means AR : Mechanisms for the testicular hypertrophy which follows hemicastration. Endocrinology **102** : 16-23, 1978
4) Putra DKH, Blackshaw AW : Morphometric studies of compensatory testicular hypertrophy in the rat after hemicastration. Aust J Biol Sci **35** : 287-293, 1982
5) Chandrasekharam VVSS, Srinivas M, Das SN, Jha P, Bajpai M, Chaki SP, Misro MM : Prepubertal human chorionic gonadotropin injection affects postpubertal germ cell maturation and androgen production in rat testis. Urology **62** : 571-574, 2003
6) Bergh A, Damber J-E, Lindgren S : Compensatory hypertrophy of the Leydig cells in hemiorchidectomized adult rats. Experientia **38** : 597-598, 1982
7) Furuya T : Oncet of compensatory hypertrophy of interstitial tissue and Leydig cells in rats hemicastrated around the time of puberty. Biol Reprod **42** : 491-498, 1990
8) England BG : Acute changes in serum LH and FSH concentrations following unilateral and bilateral orchidectomy in rats. Biol Reprod **7** : 116 (abstract 47), 1972
9) Ramirez VD, Sawyer CH : A sex difference in the rat pituitary FSH response to unilateral gonadectomy as revealed in plasma radioimmunoassays. Endocrinology **94** : 475-482, 1974
10) Gomes WR, Jain SK : Effect of unilateral and bilateral castration and cryptorchidism on serum gonadotrophins in the rat. J Endocrinol **68** : 191-196, 1976
11) Frankel AI, Mock EJ, Chapman JC : Hypophysectomy and hemivasectomy can inhibit the testicular hemicastration response of the mature rat. Biol Reprod **30** : 804-808, 1984
12) Brown JL, Chakraborty PK : Comparison of compensatory pituitary and testicular responses to hemicastration between prepubertal and mature rats. J Androl **12** : 119-125, 1991
13) Lowry OH, Rosebrough NJ, Farr AL, Randall RJ : Protein measurement with the Folin phenol reagent. J Biol Chem **193** : 265-275, 1951
14) Scatchard G : The attractions of proteins for small molecules and ions. Ann NY Acad Sci **51** : 660-672, 1949
15) Bliss CI : The Statistics of Bioassay. Academic Press, New York, 1952
16) Bliss CI : Statistics in Biology. McGraw-Hill, New York, 253-257, 1967
17) Agee J, Parsa C, Huntrakoon M : Morphologic response of rat Leydig cells to hemicastration. Archiv Androl **20** : 1-9, 1988
18) Ketelslegers JM, Hetzel WD, Sherins RJ, Catt KJ : Developmental changes in testicular gonadotropin receptors : plasma gonadotropins and plasma testosterone in the rat. Endocrinology **103** : 212-222, 1978
19) Odell WD, Swerdloff RS : Etiologies of sexual maturation : a model system based on the sexually maturing rat. Recent Prog Horm Res **32** : 245-288, 1976
20) Wahlström T, Huhtaniemi I, Hovatta O, Seppälä M : Localization of luteinizing hormone, follicle-stimulating hormone, prolactin, and their receptors in human and rat testis using immunohistochemistry and radioreceptor assay. J Clin Endocrinol Metab **57** : 825-830, 1983
21) Benahmed M, Morera AM, Chauvin MA, Peretti ED, Revol A : FSH regulates testicular steroidogenesis through Sertoli cell factors. Ann NY Acad Sci **513** : 470-472, 1987
22) Brown JL, Dahl KD, Chakraborty PK : Effects of follicular fluid administration on serum bioactive and immunoreactive FSH concentrations and compensatory testosterone secretion in hemicastrated adult rats. J Androl **12** : 221-225, 1991
23) Connell GM, Eik-Nes KB : Testosterone production by rabbit testis slices. Steroids **12** : 507-516, 1968

# ラットの思春期前後の片側去勢によるゴナドトロピン受容体の増加

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雄ラットの精巣を思春期前後の生後20、30、40日後に片側去勢して、それぞれ30日後に残された精巣を取り出し生殖腺刺激ホルモン受容体の動態を観察した。精巣重量はどの日齢で片側去勢した群でもシャムオペレーションを施した対照群との間に有意差は見られなかった。精巣の組織を間細胞と精細管とに分離してそれぞれLHとFSHのレセプターアッセイを行った。間細胞分画を用いたLHのレセプターアッセイでは20日齢と30日齢で片側去勢した群が対照群よりもLHの特異結合が1.3倍と有意に増加した。しかしLHのレセプターアッセイでの解離定数はどの日齢でも片側去勢群と対照群の間で有意差は見られなかった。精細管分画を用いたFSHのレセプターアッセイではFSHの特異結合がどの日齢で片側去勢した群でも対照群の値との間で有意差を示さなかった。スカッチャードプロットによる解析では30日齢で片側去勢することによりLH受容体の数が1.3倍に増加していた。LH受容体の親和性はどの日齢の実験群でも対照群と同じ値を示した。30日齢で片側去勢し手術後30日にLH受容体が増加したことは30日齢での片側去勢により血中のFSH濃度が上昇し、血中LHとの協働作用によりテストステロンが多量に生成されるようになりLH受容体を増加させていることが示唆された。

〈キーワード〉　ラット、思春期、精巣、片側去勢、生殖腺刺激ホルモン受容体